

ANAEROBIC BIOPROCESSING OF SUBBITUMINOUS COAL

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ABSTRACT

Subbituminous and lignite coals contain high levels of oxygen. Extensive structure reactivity studies of Wyodak coal done by us revealed that carboxy groups, along with ether linkages, are the predominant oxygen functionalities of this coal. Chemical decarboxylation to remove oxygen and upgrade the coal can be achieved at high temperatures and under such conditions the low rank coals undergo retrogressive reactions. We are developing a anaerobic microbial process to decarboxylate coal that would operate at ambient conditions. The anaerobic microbial consortia developed has resulted in decarboxylation of coal and anaerobically bioprocessed coal has exhibited an increase in H/C ratio in comparison to unprocessed coal. In this paper, we show our new results and their implications in microbial processing of coal in relation to the current bioprocessing schemes.

INTRODUCTION

The most common biological processes applied to coal conversion have been focussed on oxidative biosolubilization. The mechanism of such coal solubilization under aerobic conditions is probably dependent on the aromatic ring hydroxylations followed by ring scission. Such reaction results in oxygenated coal product which, in turn, makes the coal poorer fuel than the starting material. In addition, requirement of aeration makes the process highly energy intensive and less practical.

Anaerobic bioconversion of coal provides alternative technology for biological processing of coal. The major advantages of this approach is that it can: 1) remove oxygen from coal by decarboxylation; 2) cleave ether linkages of coal; and 3) reduce the contaminants such as sulfur from the coal. Moreover, the process is less energy intensive and requires simple engineering design. The approach to use anaerobic bacteria/enzyme promises to be most rewarding and exciting, since this involves depolymerization/solubilization of coal reductively, that is, the reduction of aromatic rings and promotion of reductive cleavages to produce hydrogenated products (Fig. 1). In other words, hydrogenation of coal via an anaerobic microbial process results in a more desirable fuel; oxidation via an aerobic microbial process results in a less desirable fuel form. Indeed, coal scientists throughout the ages have been trying to achieve this objective, that is, to find an inexpensive approach to producing a deoxygenated, hydrogen-rich coal fuel. In work done by us (Narayan, 1985; 1986 a,b; 1987; 1988; 1989) on the structure of Wyodak subbituminous coal (Figure 2) we have shown that carboxyl groups are a major oxygen functionality. They exist predominantly as carboxylate anions strongly chelating metal cations like Ca^{2+} and forming strong macromolecular crosslinks which contribute in large measure to the network polymer structure. Furthermore, the coal oligomer chains are not very long i.e. molecular weights of the coal clusters comprising the coal macromolecule have $M_n = 800-1000$ and $M_w = 2500-3500$.

Unfortunately, chemical or thermal decarboxylation can be performed only at elevated temperatures. This results in retrogressive crosslinking reactions. Indeed, Suuberg et al., (1985, 1987) have documented that low temperature crosslinking associated with low rank coal correlates well with evolution of CO_2 . This correlation has been confirmed in recent work done by Solomon and co-workers (Deshpande et al., 1988; Solomon et al., 1988, 1990). In a structural sense, what these results imply is that at temperatures needed to remove the carboxyl group, the chains are still in close proximity, and the carboxylate and hydrogen bonded crosslinks are replaced by much stronger carbon-carbon covalent crosslinks. This results in a much more intractable coal macromolecule.

In principle then, removal of the carboxyl groups at room temperature (reductive decarboxylation) would unravel the macromolecular network, resulting in a very low molecular weight coal macromolecule with increased H/C ratio. With a increased hydrophobic character, this coal could be easily cleaned and serve directly as a solid fuel source. Since the coal macromolecular network has been dismantled, this coal could be easily processed in a subsequent liquefaction step. In summary, decarboxylation of coal at ambient temperatures has the potential for developing a coal product which has better fuel value and better processing prospects.

EXPERIMENTAL

All the experiments were carried out in anaerobic pressure tubes (27 mL, Bellco Glass, Inc., Vineland, NJ) and the manipulations were performed anaerobically using sterile syringes and needles. All chemicals and gases were of analytical grade. Chemicals were obtained from Sigma Chemical Co. (St. Louis, MO). Gases (N_2 , H_2) and gas mixture N_2 - CO_2 (95:5) and H_2 - CO_2 (80:20) were obtained from Union Carbide Corp., Linde Division (Warren, MI) and passed over heated copper fillings to remove traces of O_2 .

Liquid media and solutions were prepared and sterilized under a strictly anaerobic N_2 atmosphere by methods previously described (Zeikus, et al., 1980). The phosphate buffered basal (PBB) medium (Kenealy and Zeikus, 1981) was used for all the experiments. This medium was supplemented with (per 100 mL): 1.0 mL phosphate buffer, 1.0 mL vitamin solution (Wolin et al., 1963), 0.05 g yeast extract, and 2.5 mL of 2.5% $\text{Na}_2\text{S}_9\text{H}_2\text{O}$ as well as, where added, 0.25 g supplemental carbon and energy source. Coal (Subbituminous, Wyodak) was added @ 0.15 g/10 mL media. The pH of the medium was about 7.0. Inoculum used in the present studies was obtained from a waste treatment site in Michigan and was collected and stored anaerobically at 4°C.

Initially, appropriate tubes were inoculated with mixed microbial consortia of anaerobic bacteria @ 5% and the tubes were then incubated at 37°C. Periodically the gas phase of the tubes was analyzed for CO_2 and CH_4 . Gas samples were withdrawn from the tubes with a 1.0 mL glass syringe (Container Corp., Sioux City, Iowa) equipped with a gas tight mininert syringe valve (Alltech Associates, Inc., Deerfield, MI) and a 23 gauge needle. Coal was separated from medium by centrifugation at 4,500 rpm for 10 minutes, suspended in 3 N HCl and washed with distilled water until free of acid. The coal was then vacuum dried at 70°C for 24 hours before used for elemental analysis and subjected to FT-IR analyses. Carbon dioxide and methane gas was analyzed using a Gow-Mac series 580 gas chromatograph (GOW-MAC Instrument Co., Bridgewater, NJ) equipped with a thermal conductivity detector (TCD) and cabosttpere SS column with helium as carrier gas.

RESULTS AND DISCUSSION

Microbial Non-oxidative Decarboxylation of Coal:

Subbituminous Wyodak coal was used in the present studies since this coal contains much higher levels of oxygen than bituminous coals. It has been reported that the carboxyl groups account for an estimated two thirds of this oxygen (Sandreal and Wiltsee, 1984). Microorganisms capable of reductive decarboxylation of organic compounds may have the potential of removing carboxyl oxygen from coal. We, therefore, developed appropriate enrichments for developing anaerobic mixed cultures capable of decarboxylating coal under batch conditions. Various supplemental carbon and energy sources were used to support the growth of bacteria. The tubes containing coal and an additional carbon source were inoculated with a mixed microbial consortia and incubated at 37°C. The gas phase of these tubes was analyzed for CO₂. The coal was also analyzed for carbon and hydrogen and the change in H/C ratio was calculated.

The preliminary results presented in Table 1 indicate that CO₂ was produced in all the tubes and that level of CO₂ increased over the period of incubation. The increase in H/C ratio can be attributed to loss of carbon along with oxygen. Lactate, succinate and malonate supplemented tubes showed higher levels of CO₂ from coal than those supplemented with vanillate and glutamate. It seems that these substrates supported better growth of the microbial population that has decarboxylating enzymes. Crawford and Olson (1978) used vanillate as a model compound to examine microbial decarboxylation of complex aromatic compounds. They reported non-oxidative decarboxylation of vanillate by a single enzymatic transformation and also showed, using FT-IR, removal of carboxyl groups of coal when it was incubated with *Bacillus megaterium*. Decarboxylation of succinate to propionate under anaerobic conditions has also been observed using *Selenomonas ruminantium* (Scheifinger and Wolin, 1973), *Propionibacterium pentosaceum*, *Veillonella alcalescens* (Yousten and Delwiche, 1961; Samuelov et al., 1990) and *Propionigenium modestum* (Schink and Pfennig, 1982). Decarboxylation reaction also occurs when L-glutamate is anaerobically metabolized by *Acidaminococcus fermentans*, *Peptostreptococcus asaccharolyticus*, and *Clostridium symbiosum* (Dimroth, 1987). Thus, the supplemental carbon sources used in the present study have been shown by other groups to support growth of anaerobic cultures having decarboxylases.

Based on the preliminary experiment, succinate and lactate were selected to be used as supplemental carbon sources in conducting further experiments on coal decarboxylation. From the previous experiment it was not possible to conclude whether all or any CO₂ was produced from coal. Therefore, another experiment was designed to include controls without coal to determine CO₂ production from supplemental carbon sources as well. Also, since the tubes were inoculated from an anaerobic mixed microbial consortia that contained methanogenic population, it was likely that some of CO₂ will have converted to methane especially under the long-term incubation conditions. No attempts were made to inhibit methanogenesis since the adverse effects of inhibitors of methanogens on the organisms responsible for coal decarboxylation is not known. Therefore, gas phase of these tubes were analyzed for both CO₂ and CH₄. The results obtained are summarized in Table 2 and Figures 3 and 4.

The data presented in Table 2 show that CO₂ was produced from succinate as well as lactate. However, it is important to note that levels of CO₂ produced from succinate

supplemented coal and lactate supplemented coal were higher than succinate or lactate alone indicating that additional CO_2 was produced as a result of coal decarboxylation. In addition, methane was also produced and the increased levels of methane were observed in tubes containing coal and supplemental carbon source than in tubes that contained no coal. Figures 3 and 4 show decrease in CO_2 and increase in CH_4 levels after day 9. Also the levels of CO_2 and CH_4 produced from succinate or lactate were always lower than those obtained from coal supplemented with succinate or lactate. Acetate, methanol, methylamines, formate, H_2 - CO_2 , and CO are known to serve as methanogenic substrates to different methanogenic bacteria (Jain et al., 1988). Neither succinate nor lactate is a substrate for methanogens; however, under these experimental conditions methane is expected to be produced from acetate as well as CO_2 . Succinate upon decarboxylation would be converted to propionate which in turn will be degraded to acetate by syntrophic propionate degraders. Acetate and CO_2 so produced will then be converted to methane by methanogenic bacteria. Syntrophic propionate degraders are very slow growing organisms and as a result propionate conversion to acetate is a slow reaction. It is likely that sudden increase in methane at 4 weeks time in coal supplemented with succinate may be the result of establishment of such a consortia (Figure 3). These results, however, clearly show decarboxylation of coal under anaerobic conditions. Since these experiments were carried out with mixed microbial consortia, it is not possible to hypothesize the number or type of organisms responsible for coal decarboxylation.

Energetics and Process Considerations:

One of the major problems plaguing coal bioprocesses is the consumption of coal carbon for microbial growth and maintenance. In the present decarboxylation scheme, the decarboxylation reaction is coupled to generation of an electrochemical gradient of sodium ions. This gradient can be transformed into a pH gradient that can be taken advantage of by the ATP synthase. The decarboxylation of oxaloacetate, for example, is associated with a free energy change of $\Delta G^\circ = -30 \text{ kJ } (-7.2 \text{ kcal}) \text{ mol}^{-1}$ and one could expect synthesis of 1/3 ATP per 1 CO_2 formed (Gottschalk, 1986). The uptake of 3H^+ per ATP synthesized would be in agreement with this assumption. Based on this assumption, a hypothetical model showing sodium dependent coal decarboxylation is proposed (Figure 5).

Another problem confronting coal bioprocesses particularly anaerobic is the reaction rate. In work done by us on succinate decarboxylation to propionate we used *Veillonella alcalescens* (Samuelov et al., 1990). Kinetic analysis of our results indicate that under steady-state conditions ($D=0.02 \text{ h}^{-1}$) the optimal specific rate of propionate formation from succinate was $0.252 \text{ g propionate/g cells/h}$. The non-growth related production coefficient was $0.246 \text{ g propionate/g cells/h}$. The high ratio between these two kinetic parameters indicates that the decarboxylation energy was used mainly for culture maintenance. From the steady-state rate of propionate formation the apparent *in-vivo* specific activity of decarboxylation was calculated to be $90\text{--}100 \text{ m moles/mg protein/min}$ (Samuelov, et al., 1990).

In conclusion, preliminary work reported in this paper demonstrates that decarboxylation of coal can be achieved at ambient temperature and pressure using anaerobic microbial catalyst. The ability to eliminate carboxyl groups and thereby break up of the macromolecular cross-links without the thermal retrogressive cross-linking reactions has major implications for processing of this low-rank subbituminous Wyodak coal and understanding its reactivity.

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Table 1. Coal Decarboxylation by an Anaerobic Microbial Consortium in Presence of Other Carbon Sources.

	% CO ₂ in Gas Phase After		*H/C Ratio in decarboxylated coal at 100 days
	15 days	100 days	
Coal + Succinate	9.86	16.17	1.10
Coal + Lactate	5.98	19.04	1.37
Coal + Malonate	12.72	14.65	1.22
Coal + Vanillate	1.58	9.47	1.08
Coal + Glutamate	5.38	9.69	1.14

*H/C ratio of original control = 0.92

Table 2. Decarboxylation of coal in presence of succinate or lactate as supplemental carbon source.

Substrate	Gases after 9 days	
	CO ₂ (%)	CH ₄ (mM)
Succinate	8.74	1.04
Coal + Succinate	14.63	1.99
Lactate	8.47	1.83
Coal + Lactate	16.34	6.09

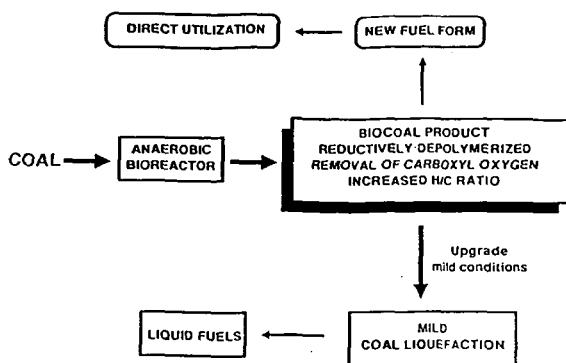
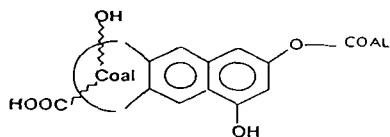
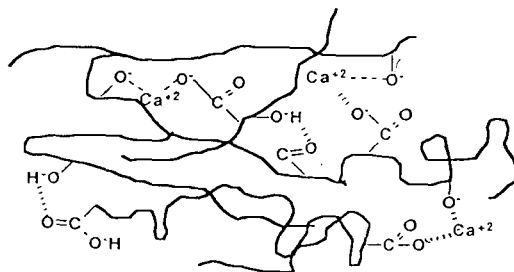


Figure 1. Schematic anaerobic bioprocess technology for conversion of coal to biocoal product and its further use.



Carboxy and dioxy groups are major oxygen functionalities



Network polymer structure due to secondary forces - hydrogen bonding and chelate crosslinks

Figure 2. Salient structural features of Wyodak subbituminous coal (Narayan, 1989).

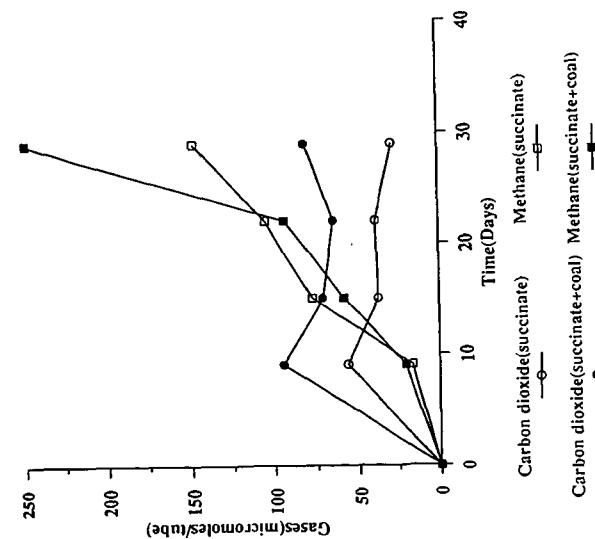


Fig.3. Production of carbon dioxide and methane from Succinate, and Coal + Succinate using anaerobic microbial consortium

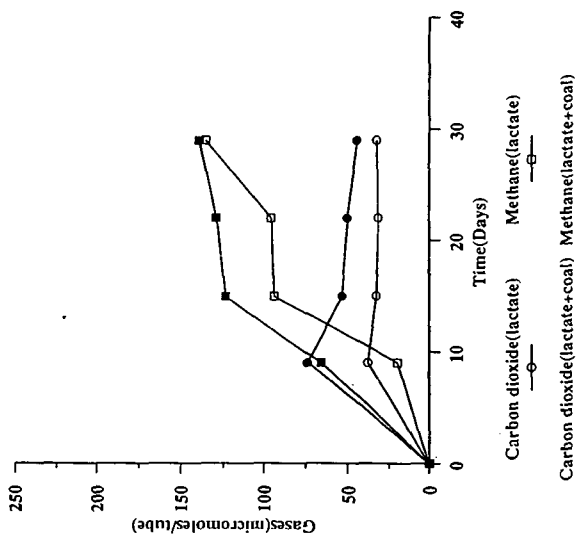


Fig.4. Production of carbon dioxide and methane from Lactate, and Coal + Lactate using anaerobic microbial consortium

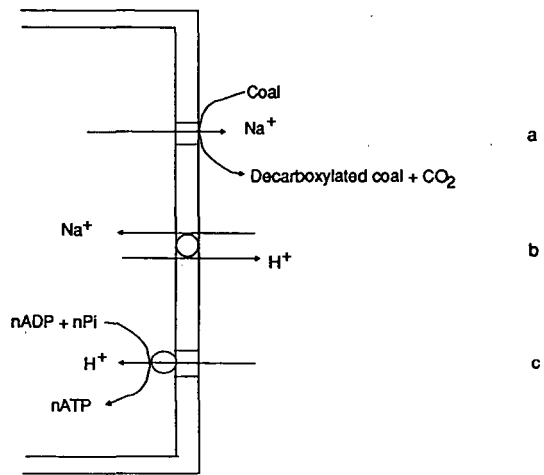


Figure 5. Hypothetical model showing sodium-dependent coal decarboxylation a: sodium translocation as coupled to the decarboxylation reaction. b: Na⁺ - H⁺ antiporter. c. Proton-translocating ATP synthase; n may be in the order of 1/3.